Amendments to the Claims:

This listing of claims replaces all prior versions and listings of claims in the application:

Listing of Claims:

1. (Currently Amended) A method comprising:

providing spectrally resolved information about light coming from different spatial locations in a sample comprising deep tissue in response to an illumination of the sample, wherein the light includes contributions from different components in the sample;

decomposing at least some of the spectrally resolved information for each of at least some of the different spatial locations into contributions from spectral estimates associated with at least some of the components in the sample; and

constructing a deep tissue image of the sample based on the decomposition to preferentially show a selected one of the components,

wherein at least a first one of the spectral estimates for a first one of the components is determined from at least part of the spectrally resolved information using an unsupervised classification technique.

2. (Original) The method of claim 1, wherein the spectrally resolved information comprises information about a set of images in which the light coming from the sample is spectrally filtered, wherein the spectral filtering for each image corresponds to a different spectral weighting function.

3. (Original) The method of claim 1, wherein the spectrally resolved information comprises information about a set of images in which light used to illuminate the sample is spectrally filtered, wherein the spectral filtering for each image corresponds to a different spectral weighting function.

4. (Original) The method of claims 2 or 3, wherein the different spatial locations correspond to common pixels in the set of images.

5. (Original) The method of claims 2 or 3, wherein the different spectral weighting functions correspond to different spectral bands.

6. (Original) The method of claims 2 or 3, wherein the set of images comprises three or more images.

7. (Original) The method of claims 2 or 3, wherein the set of images comprises four or more images.

8. (Original) The method of claim 4, wherein the information about the set of images comprises a series of values at each of the pixels, wherein each value is related to an intensity of the light coming from the sample with respect to a corresponding one of the spectral weighting functions.

9. (Original) The method of claim 1, wherein the spectrally resolved information for each spatial location comprises information corresponding to at least three different spectral weighting functions.

10. (Original) The method of claim 1, wherein the spectrally resolved information for each spatial location comprises information corresponding to at least four different spectral weighting functions.

11. (Original) The method of claim 1, wherein the spectrally resolved information comprises a spectral image cube.

12. (Original) The method of claim 1, wherein the light coming from the sample comprises fluorescence from the sample.

13. (Original) The method of claim 1, wherein the light coming from the sample comprises reflectance, phosphorescence, scattering, or Raman scattering from the sample.

14. (Original) The method of claim 1, wherein the light coming from the sample comprises transmission through the sample.

15. (Original) The method of claim 1, wherein at least one of the components relates to autofluorescence.

16. (Original) The method of claim 1, wherein at least one of the components comprises a target compound.

17. (Original) The method of claim 16, wherein the selected component is the component comprising the target compound.

18. (Original) The method of claim 16, wherein the target compound comprises a fluorescent protein or a quantum dot.

19. (Original) The method of claim 1, further comprising illuminating the sample and collecting the spectrally resolved information.

20. (Original) The method of claim 1, wherein collecting the spectrally resolved information comprises using a liquid crystal tunable spectral filter, an acousto-optical tunable spectral filter, a set of spectral filters, a spectral filter wheel, a dispersive prism, a grating, a spectrometer, or monochromator.

21. (Original) The method of claim 1, wherein the deep tissue image of the selected component comprises an image in which signal from the other components is reduced relative to signal from the selected component.

22. (Original) The method of claim 1, further comprising constructing a second deep tissue image of the sample based on the decomposition to preferentially show a second one of the components.

23. (Original) The method of claim 1, further comprising constructing a third deep tissue image of the sample based on the decomposition to preferentially show a third one of the components.

24. (Original) The method of claim 1, wherein the sample is a living organism.

25. (Original) The method of claim 1, wherein the sample is a mammal.

26. (Original) The method of claim 1, wherein at least one of the spectral estimates is an estimate of the pure spectrum of a first one of the components.

27. (Original) The method of claim 26, wherein at least some of the spectral estimates are estimates of the pure spectra for some of the components.

28. (Original) The method of claim 26, wherein the first component corresponds to autofluorescence.

29. (Original) The method of claim 26, wherein the first component corresponds to the selected component.

30. (Original) The method of claim 26, wherein the pure spectrum for the first component corresponds to the spectrally resolved information that would result if only the first component contributes to the light.

31. (Original) The method of claim 1, wherein constructing the deep tissue image based on the decomposition comprises constructing the deep tissue image based on the contributions at different spatial locations of the spectral estimate associated with the selected component.

32. (Original) The method of claim 1, wherein the decomposition is a linear decomposition.

33. (Original) The method of claim 32, wherein the decomposition comprises solving at least one component of a matrix equation in which one matrix in the equation is based on the spectrally resolved information and another matrix in the equation is based on the spectral estimates.

34. Canceled.

35. Canceled.

36. (Currently Amended) The method of claim [[35]] 1, wherein all of the spectral estimates are determined from the spectrally resolved information.

37. (Currently Amended) The method of claim 36, wherein the spectral estimates are determined from the spectrally resolved information by using [[an]] the unsupervised classification technique.

38. Canceled.

39. (Currently Amended) The method of claim [[37]] 1, wherein the unsupervised classification technique comprises averaging the spectrally resolved information for multiple ones of the spatial locations.

40. Canceled.

41. (Currently Amended) The method of claim [[35]] 1, wherein the first spectral estimate is derived from the spectrally resolved information from a first set of one or more spatial locations in which the light includes contributions from multiple ones of the components.

42. (Original) The method of claim 41, wherein the first spectral estimate is derived from the spectrally resolved information from the first set of spatial locations and a second one of the spectral estimates for a second one of the components.

43. (Original) The method of claim 42, wherein deriving the first spectral estimate comprises calculating a remainder spectrum based on the spectrally resolved information from the first set and the spectral estimate for the second component.

44. (Original) The method of claim 43, wherein the remainder spectrum is calculated at each of one or more of the spatial locations in the first set of spatial locations.

45. (Original) The method of claim 43, wherein the remainder spectrum is calculated based on an average of the spectrally resolved information in the first set of spatial locations and the spectral estimate for the second component.

46. (Original) The method of claim 42, wherein the spectral estimate for the second component is derived from the spectrally resolved information.

47. (Currently Amended) The method of claim 46, wherein the spectral estimate for the second component is determined from the spectrally resolved information by using ~~an~~ the unsupervised classification technique.

48. (Original) The method of claim 46, wherein the spectral estimate for the second component is derived from a region comprising one or more of the spatial locations, wherein the region is associated with the second component.

49. (Original) The method of claim 42, wherein deriving the first spectral estimate comprises adjusting values corresponding to the spectrally resolved information for the first set of spatial locations to remove a contribution from the second component based on the spectral estimate for the second component.

50. (Original) The method of claim 49, wherein the removed contribution is a maximal contribution.

51. (Original) The method of claim 50, wherein the maximal contribution is based on an error function analysis of signal in each spectral channel of the adjusted values.

52. (Original) The method of claim 51, wherein the error function analysis tends to maintain nonnegative signal in each spectral channel of the adjusted values.

53. (Original) The method of claim 49, wherein the values comprises a series of at least some of the values for each of the spatial locations in the first set, and wherein removing the contribution from the second component based on the spectral estimate for the second component comprises subtracting an optimized quantity of the spectral estimate for the second component from each of the series of values.

54. (Original) The method of claim 53, wherein determining the optimized quantity for at least a first of the series of values is based on minimizing an error function of a difference spectrum that includes a difference between the first series values and the quantity to be optimized multiplied by the spectral estimate for the second component, wherein the error function is minimized over the first series of values.

55. (Original) The method of claim 54, wherein the difference spectrum further includes a constant that is also optimized over the first series of values.

56. (Original) The method of claim 54, wherein the error function favors positive values of the difference spectrum over negative values of the difference spectrum.

57. (Original) The method of claim 56, wherein the error function comprises $(e^{-\Delta}+1)\Delta^2$, where Δ is the difference spectrum.

58. (Original) The method of claim 54, wherein the error function is normalized by the magnitudes of the first series of values and the spectral estimate for the second component.

59. (Currently Amended) ~~The method of claim 35~~ A method comprising:

providing spectrally resolved information about light coming from different spatial locations in a sample comprising deep tissue in response to an illumination of the sample, wherein the light includes contributions from different components in the sample;

decomposing at least some of the spectrally resolved information for each of at least some of the different spatial locations into contributions from spectral estimates associated with at least some of the components in the sample; and

constructing a deep tissue image of the sample based on the decomposition to preferentially show a selected one of the components,

wherein the decomposition comprises a first decomposition of the spectrally resolved information at multiple spatial locations into contributions from initial spectral estimates associated with at least some of the components in the sample, improving an accuracy of at least some of the initial spectral estimates based on the first decomposition, and at least a second decomposition of the spectrally resolved information at multiple spatial locations into contributions from the improved spectral estimates.

60.-67. Canceled.

68. (Currently Amended) A method comprising:

providing spectrally resolved information about light coming from different spatial locations of a biological sample in response to an illumination of the sample, wherein the light includes contributions from different components in the sample;

decomposing at least some of the spectrally resolved information for each of at least some of the different spatial locations into a contribution from a spectral estimate of a pure spectrum for at least a first one of the components in the sample; and

constructing an image of the sample based on the decomposition to preferentially show a selected one of the components,

wherein ~~the decomposition comprises deriving~~ the estimate of the pure spectrum for the first component ~~based on both~~ is derived from at least part of the spectrally resolved information corresponding to a first set of one or more of the different spatial locations and a spectral estimate of a pure spectrum for a second one of the components by using an unsupervised classification technique.

69. (Original) The method of claim 68, wherein the sample comprises deep tissue, tissue slices, cells, subdermal tissue, or a microscope slide carrying biological material.

70. (Original) The method of claim 68, wherein the decomposing comprises decomposing the spectrally resolved information for each of at least some of the different spatial locations into contributions from estimates of pure spectra for at least some of the components in the sample.

71. (Original) The method of claim 68, wherein the decomposition is a linear decomposition.

72. (Original) The method of claim 71, wherein the decomposition comprises solving at least one component of a matrix equation in which one matrix is based on the spectrally resolved information and another matrix is based on the estimate of the pure spectrum for the first component and an estimate for the pure spectrum of at least one additional component.

73. (Original) The method of claim 68, wherein the pure spectrum for the first component corresponds to the spectrally resolved information that would result if only the first component contributes to the light.

74. Canceled.

75. (Original) The method of claim 68, wherein the spectral estimate of the pure spectrum for the second component is determined from the spectrally resolved information.

76. (Currently Amended) The method of claim 75, wherein the spectral estimate of the pure spectrum for the second component is determined from the spectrally resolved information by using [[an]] <u>the</u> unsupervised classification technique.

77. Canceled.

78. (Original) The method of claim 77, wherein the unsupervised classification technique comprises averaging the spectrally resolved information for multiple ones of the spatial locations.

79. Canceled.

80. (Original) The method of claim 68, wherein the spatial locations in the first set are spatial location in which the light includes contributions from multiple ones of the components.

81. (Original) The method of claim 68, wherein deriving the spectral estimate of the pure spectrum for the first component comprises calculating a remainder spectrum based on the spectrally resolved information from the first set and the spectral estimate for the second component.

82. (Original) The method of claim 81, wherein the remainder spectrum is calculated at each of one or more of the spatial locations in the first set of spatial locations.

83. (Original) The method of claim 82, wherein the remainder spectrum is calculated based on an average of the spectrally resolved information in the first set of spatial locations and the spectral estimate for the second component.

84. (Original) The method of claim 68, wherein deriving the spectral estimate of the pure spectrum for the first component comprises adjusting values corresponding to the spectrally resolved information for the first set of spatial locations to remove a contribution from the second component based on the spectral estimate for the second component.

85. (Original) The method of claim 84, wherein the removed contribution is a maximal contribution.

86. (Original) The method of claim 85, wherein the maximal contribution is based on an error function analysis of the signal in each spectral channel of the adjusted values.

87. (Original) The method of claim 86, wherein the error function analysis tends to maintain nonnegative signal in each spectral channel of the adjusted values.

88. (Original) The method of claim 84, wherein the values comprises a series of at least some of the values for each of the spatial locations in the first set, and wherein removing the contribution from the second component based on the spectral estimate for the second component comprises subtracting an optimized quantity of the spectral estimate for the second component from each of the series of values.

89. (Original) The method of claim 88, wherein determining the optimized quantity for at least a first of the series of values is based on minimizing an error function of a difference spectrum that includes a difference between the first series values and the quantity to be optimized multiplied by the spectral estimate for the second component, wherein the error function is minimized over the first series of values.

90. (Original) The method of claim 89, wherein the difference spectrum further includes a constant that is also optimized over the first series of values.

91. (Original) The method of claim 89, wherein the error function favors positive values of the difference spectrum over negative values of the difference spectrum.

92. (Original) The method of claim 91, wherein the error function comprises $(e^{-\Delta}+1)\Delta^2$, where Δ is the difference spectrum.

93. (Original) The method of claim 89, wherein the error function is normalized by the magnitudes of the first series of values and the spectral estimate for the second component.

94. (Currently Amended) ~~The method of claim 68~~ A method comprising:

providing spectrally resolved information about light coming from different spatial locations of a biological sample in response to an illumination of the sample, wherein the light includes contributions from different components in the sample;

decomposing at least some of the spectrally resolved information for each of at least some of the different spatial locations into a contribution from a spectral estimate of a pure spectrum for at least a first one of the components in the sample; and

constructing an image of the sample based on the decomposition to preferentially show a selected one of the components,

wherein the estimate of the pure spectrum for the first component is derived from at least part of the spectrally resolved information corresponding to a first set of one or more of the different spatial locations and a spectral estimate of a pure spectrum for a second one of the components, and

wherein the decomposition comprises a first decomposition of the spectrally resolved information at multiple spatial locations into contributions from initial spectral estimates associated with at least some of the components in the sample, improving an accuracy of at least some of the initial spectral estimates based on the first decomposition, and at least a second decomposition of the spectrally resolved information at multiple spatial locations into contributions from the improved spectral estimates.

95. (Original) The method of claim 68, wherein the spectrally resolved information comprises information about a set of images in which the light coming from the sample is spectrally filtered, wherein the spectral filtering for each image corresponds to a different spectral weighting function.

96. (Original) The method of claim 68, wherein the spectrally resolved information comprises information about a set of images in which light used to illuminate the sample is spectrally filtered, wherein the spectral filtering for each image corresponds to a different spectral weighting function.

97. (Original) The method of claims 95 or 96, wherein the different spatial locations correspond to common pixels in the set of images.

98. (Original) The method of claims 95 or 96, wherein the different spectral weighting functions correspond to different spectral bands.

99. (Original) The method of claims 95 or 96, wherein the set of images comprises three or more images.

100. (Original) The method of claims 95 or 96, wherein the set of images comprises four or more images.

101. (Original) The method of claim 97, wherein the information about the set of images comprises a series of values at each of the pixels, wherein each value is related to an intensity of the light coming from the sample with respect to a corresponding one of the spectral weighting functions.

102. (Original) The method of claim 68, wherein the spectrally resolved information for each spatial location comprises information corresponding to at least three different spectral weighting functions.

103. (Original) The method of claim 68, wherein the spectrally resolved information for each spatial location comprises information corresponding to at least four different spectral weighting functions.

104. (Original) The method of claim 68, wherein the spectrally resolved information comprises a spectral image cube.

105. (Original) The method of claim 68, wherein the light coming from the sample comprises fluorescence from the sample.

106. (Original) The method of claim 68, wherein the light coming from the sample comprises reflectance, phosphorescence, scattering, or Raman scattering from the sample.

107. (Original) The method of claim 68, wherein the light coming from the sample comprises transmission through the sample.

108. (Original) The method of claim 68, wherein at least one of the components relates to autofluorescence.

109. (Original) The method of claim 68, wherein at least one of the components comprises a target compound.

110. (Original) The method of claim 109, wherein the selected component is the component comprising the target compound.

111. (Original) The method of claim 109, wherein the target compound comprises a fluorescent protein or a quantum dot.

112. (Original) The method of claim 68, further comprising illuminating the sample and collecting the spectrally resolved information.

113. (Original) The method of claim 68, wherein collecting the spectrally resolved information comprises using a liquid crystal tunable spectral filter, an acousto-optical tunable spectral filter, a set of spectral filters, a spectral filter wheel, a dispersive prism, a grating, a spectrometer, or monochromator.

114. (Original) The method of claim 68, wherein the image of the selected component comprises an image in which signal from the other components is reduced relative to signal from the selected component.

115. (Original) The method of claim 68, further comprising constructing a second image of the sample based on the decomposition to preferentially show a second one of the components.

116. (Currently Amended) An apparatus comprising:

a sample holder configured to support a deep tissue sample;

an illumination source to illuminate the sample;

a detector positioned to detect light from the sample; and

an electronic processor coupled to the detector, wherein the electronic processor ~~and~~ is configured to: (i) provide spectrally resolved information about light coming from different spatial locations of a sample, wherein the light includes contributions from different components in the sample; (ii) decompose at least some of the spectrally resolved information for each of at least some of the different spatial locations into contributions from spectral estimates associated with at least some of the components in the sample; and (iii) construct a deep tissue image of the sample based on the decomposition to preferentially show a selected one of the components, wherein the processor determines at least a first one of the spectral estimates for a first one of the components from at least part of the spectrally resolved information using an unsupervised classification technique..

117. Canceled.

118. (Currently Amended) An apparatus comprising:

a sample holder configured to support a sample;

an illumination source to illuminate the sample;

a detector positioned to detect light from the sample; and

an electronic processor coupled to the detector, wherein the electronic processor ~~and~~ is configured to: (i) provide spectrally resolved information about light coming from different

spatial locations of a sample, wherein the light includes contributions from different components in the sample; (ii) decompose at least some of the spectrally resolved information for each of at least some of the different spatial locations into a contribution from a spectral estimate of a pure spectrum for at least a first one of the components in the sample; and (iii) construct an image of the sample based on the decomposition to preferentially show a selected one of the components,

wherein the processor ~~decomposition comprises deriving~~ derives the estimate of the pure spectrum for the first component ~~based on~~ from at least part of the spectrally resolved information corresponding to a first set of one or more of the different spatial locations and a spectral estimate of a pure spectrum for a second one of the components by using an unsupervised classification technique.

119. (Currently Amended) The apparatus of claims 116, ~~117, or~~ 118, or 167, further comprising a spectral filtering means positioned between the sample and the detector.

120. (Original) The apparatus of claim 119, wherein the spectral filtering means comprises a liquid crystal tunable spectral filter, an acousto-optical tunable spectral filter, a set of spectral filters, a spectral filter wheel, a dispersive prism, a grating, a spectrometer, or monochromator.

121. (Currently Amended) The apparatus of claims 116, ~~117, or~~ 118, or 167, further comprising a spectral filtering means positioned between the illumination source and the sample.

122. (Currently Amended) The apparatus of claims 116, ~~117, or~~ 118, or 167, wherein the illumination source provides tunable excitation light.

123. (Currently Amended) Apparatus comprising a computer-readable medium storing a program that causes a processor to carry out the steps of any of claims 1, [[60]] 59, ~~and~~ 68, 94, and 167.

124.-166. Canceled.

167. (New) A method comprising:

providing spectrally resolved information about light coming from different spatial locations in a sample in response to an illumination of the sample, wherein the light includes contributions from different components in the sample;

decomposing at least some of the spectrally resolved information for each of at least some of the different spatial locations into contributions from spectral estimates associated with at least some of the components in the sample; and

constructing an image of the sample based on the decomposition to preferentially show a selected one of the components,

wherein the decomposition comprises a first decomposition of the spectrally resolved information at multiple spatial locations into contributions from initial spectral estimates associated with at least some of the components in the sample, improving an accuracy of at least some of the initial spectral estimates based on the first decomposition, and at least a second decomposition of the spectrally resolved information at multiple spatial locations into contributions from the improved spectral estimates.

168. (New) An apparatus comprising:

a sample holder;

an illumination source to illuminate the sample;

a detector positioned to detect light from the sample; and

an electronic processor coupled to the detector, wherein the electronic processor is configured to: (i) provide spectrally resolved information about light coming from different spatial locations of a sample, wherein the light includes contributions from different components in the sample; (ii) decompose at least some of the spectrally resolved information for each of at least some of the different spatial locations into contributions from spectral estimates associated with at least some of the components in the sample; and (iii)

construct an image of the sample based on the decomposition to preferentially show a selected one of the components,

wherein the decomposition by the electronic processor comprises a first decomposition of the spectrally resolved information at multiple spatial locations into contributions from initial spectral estimates associated with at least some of the components in the sample, improving an accuracy of at least some of the initial spectral estimates based on the first decomposition, and at least a second decomposition of the spectrally resolved information at multiple spatial locations into contributions from the improved spectral estimates.